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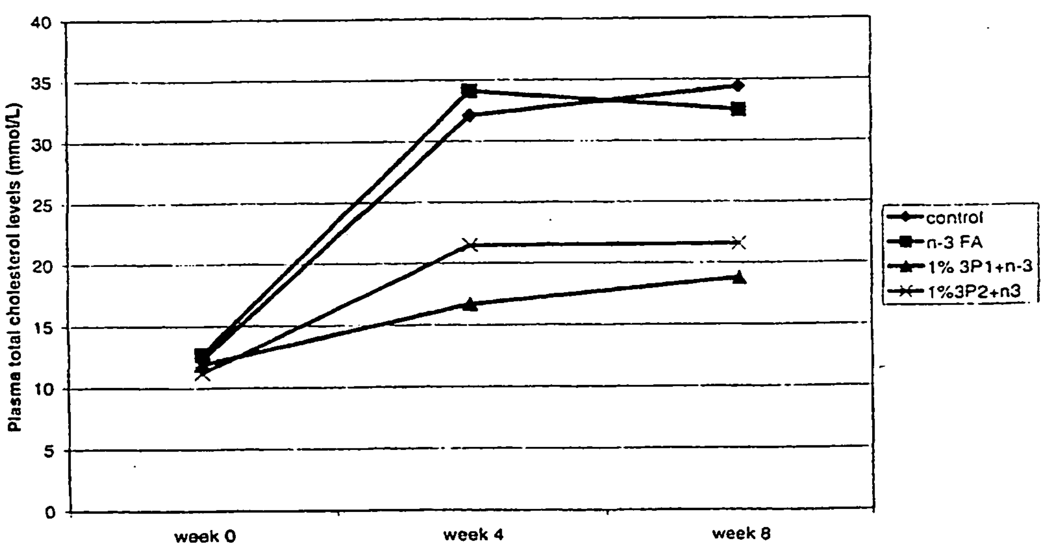


(57) Abstract

A composition for use in preventing and treating cardiovascular disease and other disorders comprises one or more phytosterols, phytostanols or mixtures of both, and one or more omega-3 polyunsaturated fatty acids or derivatives thereof.

COMPOSITIONS COMPRISING PHYTOSTEROL, PHYTOSTANOL OR MIXTURES OF BOTH AND OMEGA-3 FATTY ACIDS OR DERIVATIVES THEREOF AND USE OF THE COMPOSITION IN TREATING OR PREVENTING CARDIOVASCULAR DISEASE AND OTHER DISORDERS

FIELD OF THE INVENTION

This present invention relates to the field of preventing and treating cardiovascular disease and other disorders using phytosterol-based compositions.

BACKGROUND OF THE INVENTION

While recent advances in science and technology are helping to improve quality and add years to human life, the prevention of atherosclerosis, the underlying cause of cardiovascular disease ("CVD") has not been sufficiently addressed. Research to date suggest that cholesterol may play a role in atherosclerosis by forming atherosclerotic plaques in blood vessels, ultimately cutting off blood supply to the heart muscle or alternatively to the brain or limbs, depending on the location of the plaque in the arterial tree (1,2). Overviews have indicated that a 1% reduction in a person's total serum cholesterol yields a 2% reduction in risk of a coronary artery event (3). Statistically, a 10% decrease in average serum cholesterol (e.g. from 6.0 mmol/L to 5.3 mmol/L) may result in the prevention of 100,000 deaths in the United States annually (4).

Sterols are naturally occurring triterpenoids that perform many critical cellular functions. Phytosterols such as campesterol, stigmasterol and beta-sitosterol in plants, ergosterol in fungi and cholesterol in animals are each primary components of cellular and sub-cellular membranes in their respective cell types. The dietary source of phytosterols in humans comes from plant materials i.e. vegetables and plant oils. The estimated daily phytosterol content in the conventional western-type diet is approximately 60-80 milligrams in contrast to a vegetarian diet which would provide about 500 milligrams per day.

The present invention further comprises a method of treating or preventing CVD and other disorders such as diabetes type II, visceral obesity and hypertension in animals, including humans, by administering to the animal a composition which comprises one or more phytosterols, phytostanols or mixtures of both and one or more omega-3 polyunsaturated fatty acids or derivatives thereof.

The composition of the present invention has marked advantages over the phytosterol/stanol compositions previously known and described in the art. In particular and quite surprisingly, it has been found that there is an additive or synergistic effect between the phytosterol/stanol component and the omega-3 polyunsaturated fatty acid component of the composition on the absorption, catabolism and excretion of cholesterol and on the catabolism of triglycerides. These effects and other advantages are described in more detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing total cholesterol levels in plasma of 4 groups of the animals at baseline and during the experimental course (weeks 4 and 8); and

Figure 2 is a graph showing triglyceride levels in plasma of 4 groups of the animals at baseline and during the experimental course (weeks 4 and 8).

PREFERRED EMBODIMENTS OF THE INVENTION

According to one aspect of the present invention, there is provided a composition suitable for use alone or for incorporation into foods, beverages, pharmaceuticals, nutraceuticals and the like which comprises one or more phytosterols, phytostanols or mixtures of both and one or more omega-3 polyunsaturated fatty acids or derivatives thereof. This composition has been found to have significant effects on the prevention and treatment of CVD and other disorders. In order to understand the possible mechanism of the synergy between the phytosterol/stanol component and the omega-3 polyunsaturated fatty acid

cholesterol rich microparticle complexes with apoprotein B ("apo B"). In the enterocytes, phytosterols compete with cholesterol for apo B forming more lipophilic, apolar apo B complexes which cause shuttle inhibition and decrease lymphatic cholesterol content. If there is a decrease or absence of cellular synthesis of apo B, both serum phytosterol and cholesterols levels are low (i.e. diabetes type II, abetalipoproteinemia, and hypothyroidism). Changes in enterocyte shuttle selectivity, presumably due to an apo B mutation, could lead to high phytosterol and cholesterol levels (primary familial hypercholesterolemia and sitosterolemia).

As used herein, the term "systemic effect" refers to the effect of phytosterols on cholesterol bile acid synthesis, enterocyte and biliary cholesterol excretion. bile acid excretion, and changes in enzyme kinetics and cholesterol transport between various compartments within the body i.e. primary compartments such as the liver and enterocytes, secondary compartments such as organs, tissues and cells and tertiary compartments such as endothelial cells, monocytes and atherosclerotic plaque.

As in the enterocyte shuttle, phytosterols compete with cholesterol in the hepatic cells of the liver for elimination. In contrast with the enterocyte shuttle, however, the elimination of phytosterol via the bile route is faster than cholesterol. Correspondingly, the endogenous phytosterol pool size is low compared to cholesterol due to the combination of poor phytosterol intestinal absorption and faster biliary excretion.

The effects of omega-3 polyunsaturated fatty acids ("omega-3 PUFAs") on cholesterol levels were appreciated after comparison of saturated fats and unsaturated fats of vegetable origin on CVD. In feeding trials, the replacement of saturated fat and cholesterol in the diet by vegetable-based polyunsaturated fats caused changes that were associated with a reduced risk of CVD (13). These changes included marked reduction in low density lipoprotein ("LDL") cholesterol and very low density lipoprotein ("VLDL") cholesterol concentrations (14 and 15). The mechanism by which polyunsaturated fats reduce plasma cholesterol levels is still poorly understood, however, it has been suggested that it may be due to a decrease in cholesterol absorption in the gut lumen, a reduction in cholesterol synthesis in the body, a shift in cholesterol content from the plasma to other body compartments (the systemic effect), a change in the rate of

subsequently taken up by tissue where they are either oxidized to generate energy or re-esterified in triglycerides for storage (adipose tissue). Further, LPL has a role as a ligand for receptors, such as the LDL receptor-related protein ("LRP") and the VLDL receptor and as such contributes to the removal of lipoproteins from the circulation. Accordingly when LPL activity or synthesis is diminished for any reason, profound perturbations in plasma lipid concentrations may ensue.

Whereas LPL is a protagonist in the metabolism of triglycerides, apo C-II can be considered as an antagonist. Apo C-III is a 79 amino acid glycoprotein produced predominantly in the liver and the intestine. As a major component of plasma chylomicrons and VLDL, apo C-III delays the catabolism of these triglyceride-rich lipoproteins as well as the subsequent removal of the remnant particles from the plasma. In vitro, apo C-III has been shown to inhibit hydrolysis of triglycerides by LPL (22 and 23). It also inhibits the apo-E mediated clearance of lipoproteins by liver cells (24 and 25).

It has been found that fatty acids alter the transcription of the of the apo C-III and LPL genes via the peroxisome proliferator activated receptor ("PPAR") (26 and 27). Further studies show that treatment of hypertriglyceridemic patients with fibrates and omega-3 PUFAs (fish oil) results in the reduction of plasma triglyceride levels (28). Clinical reports have indicated that this hypotriglyceridemic effect is accompanied by both a decrease in the plasma concentration and synthesis rate of apo C-III (29) and an increase in LPL activity (30).

This facilitation of triglyceride catabolism by omega-3 PUFAs partly explains the desirable rise in high density lipoprotein ("HDL2") cholesterol, which is augmented by the partial inhibition of the lipid transfer protein (31).

Effect of Omega-3 PUFA on Hepatic Lipid Metabolism

Omega-3 PUFAs have been reported to alter hepatic lipid metabolism by several loci including:

chylomicrons and chylomicron remnants and catabolism of triglycerides and also indirectly via cholesterol transfer protein transfer of HDL cholesterol.

In other words, it is very likely that this synergy between phytosterols/phytostanols and omega-3 PUFAs is based upon the relative enrichment by phytosterols/phytostanols of chylomicron and chylomicron remnants with t riglycerides (resulting in a decrease in the chylomicron cholesterol/triglyceride ratio) coupled with the increase by omega-3 PUFAs in the catabolism of triglycerides. This co-effect results in a decrease in postprandial lipaemia in humans by altering chlymicron composition, turnover and atherogenicity.

Phytosterols/Phytostanols

As used herein, the term "phytosterol" includes all phytosterols without limitation, for example: sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized forms and derivatives thereof, including isomers. The term "phytostanol" includes all saturated or hydrogenated phytosterols and all natural or synthesized forms and derivatives thereof, including isomers. It is to be understood that modifications to the phytosterols and phytostanols i.e. to include side chains also falls within the purview of this invention. It is also to be understood that this invention is not limited to any particular combination of phytosterols and/or phytostanols forming a composition. In other words, any phytosterol or phytostanol alone or in combination with other phytosterols and phytostanols in varying ratios as required depending on the nature of the ultimate formulation may incorporated with the omega-3 PUFAs. For example, the composition described in PCT/CA95/00555 which comprises no more than 70% by weight beta-sitosterol, at least 10% by weight campesterol and stigmastanol may be used within the scope of the present invention.

The phytosterols and phytostanols for use in this invention may be procured from a variety of natural sources. For example, they may be obtained from the processing of plant oils (including aquatic plants) such as corn oil and other vegetable oils, wheat germ oil, soy extract, rice extract, rice bran, rapeseed oil, sesame oil and fish oils. Without limiting the generality of the foregoing, it is to be understood that there are other sources of phytosterols and phytostanols such as marine animals from which the composition of the present invention may be prepared. US Patent Serial No. 4,420,427 teaches the

the number of carbon atoms contained in the group R2: the branching typically refers, but is not limited to, one or more methyl group side chains (branches); or R2 is an unbranched or branched unsaturated alkyl group, represented by the formula CnH2n-2m, where n=1-25 is the number of carbon atoms in R2 and m=degree of unsaturation; or

c) a tricarboxylic acid represented by the formula:

HOOC——R3——COOH

COOH

wherein, in this formula:

R3 is a branched saturated alkyl group represented by -CnH2n-1- where n=1-25 is the number of carbon atoms contained in the group R3; the branching typically refers, but is not limited to, one or more methyl group side chains (branches); or R3 is a branched unsaturated alkyl group, represented by CnH2n-2m-1- wherein n=1-25 is the number of carbon atoms in R3 and m= the degree of unsaturation; or

d) a mono-. di-, or tricarboxylic acid as defined above, which may contain one, two or three hydroxyl groups in the molecule.

In a preferred form, the aliphatic acid is either a straight-chain or branched unsaturated or saturated fatty acid selected. inter alia, from the following list:
valeric acid, isovaleric acid, sorbic acid, isocaproic acid, lauric acid, myrestic acid, palmitic acid, stearic acid, caproic acid, ascorbic acid, arachidic acid, behenic acid, hexacosanoic acid, octacosanoic acid, pentadecanoic acid. erucic acid, linoleic acid, linolenic acid, arachidonic acid. acetic acid. citric acid. tartaric acid, palmitoleic acid and oleic acid. The most preferable fatty acids within the scope of the present invention are linoleic acid, linolenic acid and arachidonic acid which may be obtained from natural sources such as safflower oil, sunflower oil, olive oil and corn oil (linoleic acid), safflower oil, sunflower oil, olive oil and jojoba oil (linolenic acid and arachidonic acid) and rapeseed oil (erucic acid).

synthesized using microalgae as the source material. In one preferred form, marine fish oil may be mixed directly with the phytosterol and/or stanol components to form the composition of the present invention. The marine oil may be extracted by techniques known in the art from, inter alia: finfish such as cod, salmon, tuna, herring, halibut, shark, catfish, pollock, dogfish, anchovy, mackerel, trout, and eel; animals such as seals and whales; crustaceans such as crabs, clams and lobster; mollusks and the like.

Without limiting the generality of the foregoing, the most preferred marine sources of omega-3 PUFAs are as follows:

Source	Grams, Omega-3/100 calories*
fish oil capsules	2.86
salmon (sockeye)	1.71
tuna	1.22
salmon (pink)	1.15
shark (spiny dogfish	1.14
halibut	1.13
anchovy	1.10
salmon (Atlantic)	1.08
mackerel (Atlantic)	1.08
salmon (Pacific)	1.03
spanish sardine	0.91
trout (rainbow)	0.86
mackerel (Pacific)	0.85
swordfish (herring)	0.75

* (41)

Alternatively, plant sources of omega-3 PUFAs may be used. The great advantage of plant sources is reduced odour as compared to some marine sources. Plant sources include, but are not limited to, plant oils such as hemp oil, flax seed oil and corn oil as well as soy.

Other Components

Optionally, the composition of the present invention comprising phytosterol and/or phytostanol with omega-3 PUFAs may be combined with other components to enhance further the therapeutic and dietary efficacy. For example, the composition may comprise one or more of the following:

saturated fatty acids; other PUFAs; short, medium, long or very long chain fatty acids (saturated or unsaturated); neutral fats; cholesterol; esters and triacylglycerols.

Delivery Systems

Although it is fully contemplated within the scope of the present invention that the compositions may be administered to animals, particularly humans, directly and without any further modification, it is possible to take further steps to enhance delivery and ensure even distribution throughout the food, beverage, pharmaceutical, nutraceutical and the like to which they are added. Such enhancement may be achieved by a number of suitable means such as, for example, solubilizing or dispersing the elements of the composition to form emulsions, solutions and dispersions or self-emulsifying systems; lyophilizing, spray drying, controlled precipitating, or a combination thereof; forming solid dispersions, suspensions, hydrated lipid systems; forming inclusion complexations with cyclodextrins; and using hydrotopes and formulations with bile acids and their derivatives.

Each of the techniques which may be used in accordance with the present invention are described below.

Emulsions

Emulsions are finely divided or colloidal dispersions comprising two immiscible phases, e.g. oil and water, one of which (the internal or discontinuous phase) is dispersed as droplets within the other (external or discontinuous phase). Thus an oil-in-water emulsion consists of oil as the internal phase, and water as the discontinuous or external phase, the water-in-oil emulsion being the opposite. A wide variety of emulsified systems may be formed which comprise the compositions including standard emulsions, microemulsions and those systems which are self-emulsifying (emulsify on exposure to agitated aqueous fluids such as gastric or intestinal fluids).

butylated hydroxytoluene, hydroquinone, nordihydroguaiaretic acid and alpha-tocopherol. Suitable preservatives, pH adjustment agents, and buffers, chelating agents, osmotic agents, colours and flavouring agents are discussed hereinbelow under "Supensions", but are equally applicable with respect to the formation of emulsions.

The general preparation of emulsions is as follows: the two phases (oil and water) are separately heated to an appropriate temperature, the same in both cases, generally 5-10°C above the melting point of the highest melting ingredients in the case of a solid or semi-solid oil, or where the oil phase is liquid, a suitable temperature as determined by routine experimentation). Water-soluble components are dissolved in the aqueous (water) phase and oil-soluble components, are dissolved in the oil phase. To create an oil-in water emulsion, the oil phase is vigorously mixed into the aqueous phase to create a suitable dispersion and the product is allowed to cool at a controlled rate with stirring. A water-in-oil emulsion is formed in the opposite fashion i.e. the water phase is added to the oil phase. When hydrophilic colloids are a part of the system as emulsion stabilizers, a phase inversion technique may be employed whereby the colloid is mixed into the oil phase rather than the aqueous phase, prior to addition to the aqueous phase. In using any phytosterol or phytostanol composition, it is preferred to add these to the oil phase prior to heating.

Microemulsions, characterized by a particle size at least an order of magnitude smaller (10-100 nm) than standard emulsions and defined as "a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid" (42), may also be formed comprising phytosterol or phytostanol compositions. In a preferred form, the microemulsion comprises a surfactant or surfactant mixture, a co-surfactant (usually a short chain alcohol) the chosen phytosterol or phytostanol and omega-3 PUFAs, water and optionally other additives.

This system has several advantages as a delivery system for the compositions of the present invention. Firstly, microemulsions tend to be created spontaneously, that is, without the degree of vigorous mixing required to form standard emulsions. From a commercial perspective, this simplifies the manufacturing process. Secondly, microemulsions may be sterilized using microfiltration techniques without breaking the

Suitable solubilizing agents include all food grade oils such as plant oils. marine oils (such as fish oil) and vegetable oils, monoglycerides, diglycerides, triglycerides, tocopherols and the like and mixtures thereof.

Self-Emulsifying Systems

The compositions of the present invention may be mixed with appropriate excipients, for example, surfactants, emulsion stabilizers (described above) and the like, heated (if necessary) and cooled to form a semi-solid product capable of forming a spontaneous emulsion on contact with aqueous media. This semi-solid product may be used in numerous other forms such as filler material in two-piece hard or soft gelatin capsules, or may be adapted for use in other delivery systems.

Solid Dispersions

An alternative means of further increasing the solubility/dispersability of the compositions of the present invention involves the use of solid dispersion systems. These dispersions may include molecular solutions (eutectics), physical dispersions or a combination of both.

For example, solid dispersions may typically be prepared by utilizing water-soluble polymers as carriers. Without limitation, these carriers may include, either alone or in combination: solid grade polyethylene glycols (PEG's), with or without the addition of liquid grade PEG's; polyvinylpyrrolidones or their co-polymers with vinyl acetate and cellulose ethers and esters. Other excipients. such as additional members of the glycol family e.g. propylene glycol, polyols, e.g. glycerol etc.. may also be included in the dispersions.

Solid dispersions may be prepared by a number of ways which are familiar to those in the art. These include, without limitation, the following methods:

(a) fusing the ingredients, followed by controlled cooling to allow solidification and subsequent mechanical grinding to produce a suitable powder. Alternatively, the molten (fused) dispersion may be sprayed into a stream of cooled air in a spray drier to form solid particles (prilling) or passed through an extruder and

nonoxynol-10, polysorbate 60, polysorbate 80, polysorbate 40, poloxamer 235, polysorbate 20 and poloxamer 188; anionic surfactants such as sodium lauryl sulfate and docusate sodium; fatty acids, salts of fatty acids, other fatty acid esters, and mixtures thereof.

Agents/buffers for pH adjustment include citric acid and its salts, tartaric acid and its salts, phosphoric acid and its salts, acetic acid and its salts, hydrochloric acid, sodium hydroxide and sodium bicarbonate. Suitable chelating agents include edetates (disodium, calcium disodium and the like), citric acid and tartaric acid. Suitable antioxidants include ascorbic acid and its salts, ascorbyl palmitate, tocopherols (especially alpha-tocopherol), butylated hydroxytoluene, butylated hydroxyanisole, sodium bisulfite and metabisulfite. Suitable osmotic agents include monovalent, divalent and trivalent electrolytes, monosaccharides and disaccharides. Suitable preservatives include parabens (Me, Et, Pr, Bu and mixtures thereof), sorbic acid, thimerosal, quaternary ammonium salts, benzyl alcohol, benzoic acid, chlorhexidine gluconate and phenylethanol. Colours and flavours may be added as desired and may be selected from all natural, nature-identical and synthetic varieties.

Hydrated Lipid Systems

In a further embodiment of the present invention, the solubility/dispersability of the compositions of the present invention may be further enhanced by the formation of phospholipid systems such as liposomes and other hydrated lipid phases, by physical inclusion. This inclusion refers to the entrapment of molecules without forming a covalent bond and is widely used to improve the solubility and subsequent dissolution of active ingredients.

Hydrated lipid systems, including liposomes, can be prepared using a variety of lipid and lipid mixtures, including phospholipids such as phosphatidylcholine (lecithin), phosphodiglyceride and sphingolipids, glycolipids, and the like. The lipids may preferably be used in combination with a charge bearing substances such as charge-bearing phospholipids, fatty acids, and potassium and sodium salts thereof in order to stabilize the resultant lipid systems. A typical process of forming liposomes is as follows:

1) dispersion of lipid or lipids and the phytosterols and/or phytostanols along with the

sub-units and having a toroidal cylindrical spatial configuration. Commonly available members of this group comprise molecules containing six (alpha-cyclodextrin), seven (beta-cyclodextrin) and eight (gamma-cylcodextrin) glucopyranose molcules, with the polar (hydrophilic) hydroxyl groups oriented to the outside of the structure and the apolar (lipophilic) skeletal carbons and ethereal oxygens lining the interior cavity of the toroid. This cavity is capable of accomodating (hosting) the lipophilic moiety of an active ingredient (the guest molecule, here the composition of the present invention) by bonding in a non-covalent manner to form an inclusion complex.

The external hydroxyl substituents of the cyclodextrin molecule may be modified to form derivatives having improved solubility in aqueous media along with other desired enhancements, such as lowered toxicity, etc.. Examples of such derivatives are: alkylated derivatives such as 2,6-dimethyl-beta-cyclodextrin; hydroxyalkylated derivatives such as hydroxypropyl-beta-cyclodextrin; branched derivatives such as diglucosly-beta-cyclodextrin; sulfoalkyl derivatives such as sulfobutylether-beta-cyclodextrin; and carboxymethylated derivatives such as carboxymethyl-beta-cylcodextrin. Other types of chemical modifications, known to those in the art, are also included within the scope of this invention.

The cyclodextrin complex often confers properties of improved solubility, dispersability, stability (chemical, physical and microbiological), bioavailability and decreased toxicity on the guest molecule (here, the derivative of the present invention).

There are a number of ways known in the art to produce a cyclodextrin complex. Complexes may be produced, for example, by using the following basic methods: stirring the constituents of the composition into an aqueous or mixed aqueous-organic solution of the cyclodextrin, with or without heating; kneading, slurrying or mixing the cyclodextrin and the present composition in a suitable device with the addition of an appropriate quantity of aqueous, organic or mixed aqueous-organic liquid, with or without heating; or by physical admixture the cylcodextrin and the composition of the present invention using a suitable mixing device. Isolation of the inclusion complex so formed may be achieved by co-precipitation, filtration and drying; extrusion/ spheronisation and drying; subdivision of the moist mass and drying; spray drying; lyophilization or by other suitable techniques

acrylate polymers and their derivatives (e.g. appropriate members of the Eudragit™ series), ethylcellulose or combinations thereof. Additional excipients may be added to the coating formulation to modify membrane functionality or to aid in the coating process (e.g. surfactants, plasticisers, channeling agents, permeability modifiers and the like). Coating formulation vehicles may comprise aqueous or organic systems, or mixtures of both.

Hydrotropic Complexation

Compounds which are capable of opening up the water structure associated with hydrophobic (lipophilic) and other molecules are referred to as hydrotropes. These compounds may be used to enhance further the aqueous solubility of the compositions. Examples of hydrotopes include, inter alia, sodium benzoate, sodium hydroxybenzoates, sodium salicylate, nicotinamide, sodium nicotinate, sodium gentisate, gentisic acid ethanolamide, sodium toluates, sodium aminobenzoates, sodium anthranilate, sodium butylmonoglycolsulfate, resorcinol and the like.

Complex formation, which is non-covalent in nature, may be achieved by mixing the composition and the hydrotope or mixtures thereof in a suitable liquid vehicle, which may be aqueous, organic or a combination of both. Additional excipients such as surfactants, polyols, disaccharides etc.. may be added to facilitate complexation or to aid in dispersability. The resultant complex is isolated as a dry powder by any process known in the art (co-precipitation and drying, evaporation of the liquid vehicle, spray drying, lyophilization etc..). Particle size may be reduced by any standard technique such as those described previously herein, if desired. The resultant hydrotope complex may be used without further modification or may be compounded into a variety of other formulations or vehicles as required.

Methods of Use

The composition of the present invention may be administered to animals, in particular humans, directly and without further modification or alternatively may be incorporated into various vehicles as described further below in order to treat an/or prevent CVD, its underlying conditions as well as other disorders such as diabetes type II, hypertension and visceral obesity. In populations which are considered "high-risk" for CVD, it is

2) Foods/Beverages/Nutraceuticals:

In another form of the present invention, the composition of the present invention may be incorporated into foods, beverages and nutraceuticals, including, without limitation, the following:

1) Dairy Products –such as cheeses, butter, milk and other dairy beverages, spreads and dairy mixes, ice cream and yoghurt;

2) Fat-Based Products–such as margarines, spreads, mayonnaise, shortenings, cooking and frying oils and dressings;

3) Cereal-Based Products–comprising grains (for example, bread and pastas) whether these goods are cooked, baked or otherwise processed;

4) Confectionaries–such as chocolate, candies, chewing gum, desserts, non-dairy toppings (for example Cool Whip™), sorbets, icings and other fillings;

5) Beverages– whether alcoholic or non-alcoholic and including colas and other soft drinks, juices, dietary supplement and meal replacement drinks such as those sold under the trade-marks Boost™ and Ensure™; and

6) Miscellaneous Products–including eggs, processed foods such as soups, pre-prepared pasta sauces, pre-formed meals and the like.

The composition of the present invention may be incorporated directly and without further modification into the food, nutraceutical or beverage by techniques such as mixing, infusion, injection, blending, immersion, spraying and kneading. Alternatively, the composition may be applied directly onto a food or into a beverage by the consumer prior to ingestion. These are simple and economical modes of delivery.

Measurements of plasma cholesterol and triglycerides levels:

The animals were bled from the tail at the outset and during the experimental course as described previously (47, 48). Plasma was separated by centrifugation and used for the measurement of total cholesterol and triglycerides concentrations using an enzymatic method in the clinical laboratory at the St. Paul's Hospital, Vancouver, BC. Plasma lipid measurements were performed in a blind fashion.

Statistical analysis:

One way ANOVA followed by the application of Tukey test was performed to detect statistically significant differences between the results of all 4 groups of the mice by using SPSS software.

Results

Total free fatty acids and proxide values of the experimental diets:

Auto-oxidation of the experimental diets were evaluated just after preparation and 2 weeks later by measuring total free fatty acids and peroxide value. The results are summarized in Table 1. As is evident, the 2-week storage of prepared diets was not associated with auto-oxidation. Therefore, we decided to prepare the experimental diets every other week.

Body weight:

The animals' body weights were measured weekly at the baseline and during the experimental course. Table 2 demonstrates the weekly mean body weights of all 4 experimental groups of the mice. The data indicate that all groups of mice have had a comparable body weight gain during the experimental course.

Plasma lipid profiles:

Figure 1 shows total cholesterol levels in plasma of 4 groups of the animals at baseline and during the experimental course (weeks 4 and 8). All mice had comparable total cholesterol concentrations at baseline. The Western-type diet markedly increased plasma total cholesterol concentrations (control group). Addition of 1% (w/w) n-3 fatty acid did not decrease plasma total cholesterol levels as compared to controls. One other hand, combination of n-3 fatty acid and plant sterols

Group#	Baseline	W1	%increase	W2	%increase	W3	%increase
1	19.1	23.4	22.5	24.6	28.8	26.6	39.3
	19	23.3	22.6	25.3	33.2	26.6	40.0
Controls	19.5	22.8	16.9	23.4	20.0	25.1	28.7
	19.6	22.3	13.8	23.8	21.4	25.7	31.1
	20.1	22.5	11.9	23.7	17.9	25.5	26.9
	21.8	24	10.1	25.4	16.5	27.2	24.8
	19.6	24.3	24.0	25.3	29.1	26.8	36.7
	20.4	23	12.7	25.8	26.5	27.2	33.3
Mean	**19.9**	**23.2**	**16.8**	**24.7**	**24.2**	**26.3**	**32.6**
Sd	**0.9**	**0.7**	**5.5**	**0.9**	**6.0**	**0.8**	**5.7**
2	17.6	21.5	22.2	23.9	35.8	25	42.0
	20.7	23.7	14.5	25.9	25.1	27.8	34.3
1% n-3	19.6	23.5	19.9	24.7	26.0	25.6	30.6
Fatty acids	18.5	22.3	20.5	23.8	28.6	25.2	36.2
	21.1	24.4	15.6	26.1	23.7	27.1	28.4
	19.5	22.9	17.4	24.6	26.2	26.2	34.4
	19.6	24.5	25.0	25.6	30.6	27.4	39.8
	21	24.8	18.1	25.9	23.3	28	33.3
Mean	**19.7**	**23.5**	**19.2**	**25.1**	**27.4**	**26.5**	**34.9**
Sd	**1.2**	**1.2**	**3.5**	**0.9**	**4.2**	**1.2**	**4.5**
3	20.8	23.3	12.0	24.8	19.2	27	29.8
	15.4	19.3	25.3	21.7	40.9	23.6	53.2
1% n-3	18.2	22	20.9	23.9	31.3	26.8	47.3
Fatty acids	19.9	23.2	16.6	24.6	23.6	26.6	33.7
+	20.7	23	11.1	24.8	19.8	26.7	29.0
1% FCP-3P1	22.9	24.9	8.7	26.5	15.7	28.2	23.1
Mean	**19.7**	**22.6**	**15.8**	**24.4**	**25.1**	**26.5**	**36.0**
Sd	**2.6**	**1.9**	**6.4**	**1.6**	**9.4**	**1.5**	**11.7**
4	20.6	23.2	12.6	23.8	15.5	26.1	26.7
1% n-3	19.2	23.5	22.4	24.7	28.6	26.8	39.6
fatty acids	20	24.2	21.0	25.4	27.0	27.7	38.5
+	20.5	23.2	13.2	25.3	23.4	26.8	30.7
1% FCP-3P2	19.6	23	17.3	24.6	25.5	26.3	34.2
	19.3	22.9	18.7	24.9	29.0	26.2	35.8
Mean	**19.9**	**23.3**	**17.5**	**24.8**	**24.9**	**26.7**	**34.2**
Sd	**0.6**	**0.5**	**4.0**	**0.6**	**5.0**	**0.6**	**4.9**

Group#	W7	%increase	W8	%increase	W9	%increase
1	28.3	48.2	28.8	50.8	28.5	49.2
	28.5	50.0	28.9	52.1	29.2	53.7
Controls	27.1	39.0	28.7	47.2	29.1	49.2
	26.6	35.7	27.6	40.8	27.6	40.8
	26.6	32.3	27.1	34.8	27.9	38.8
	27.9	28.0	28.4	30.3	28.5	30.7
	29.4	50.0	30.6	56.1	30.9	57.7
	30.2	48.0	30.4	49.0	30.3	48.5
Mean	**28.1**	**41.4**	**28.8**	**45.1**	**29.0**	**46.1**
Sd	**1.3**	**8.8**	**1.2**	**9.0**	**1.1**	**8.7**
2	27.7	57.4	29	64.8	29.4	67.0
	30.2	45.9	31	49.8	31.4	51.7
1% n-3	27.6	40.8	28.1	43.4	27.9	42.3
Fatty acids	24.9	34.6	24.5	32.4	24.4	31.9
	31	46.9	31.2	47.9	31.3	48.3
	29.5	51.3	29.5	51.3	29	48.7
	29.4	50.0	30.6	56.1	30.8	57.1
	29.8	41.9	30.5	45.2	30.6	45.7
Mean	**28.8**	**46.1**	**29.3**	**48.9**	**29.4**	**49.1**
Sd	**1.9**	**7.1**	**2.2**	**9.5**	**2.3**	**10.3**
3	30.5	46.6	30.5	46.6	30.6	47.1
	26.6	72.7	27.2	76.6	27.6	79.2
1% n-3	28.7	57.7	28.8	58.2	29.2	60.4
Fatty acids	28.8	44.7	28.9	45.2	28.7	44.2
+	28.6	38.2	29.5	42.5	29.8	44.0
1% FCP-3P1	30.2	31.9	31.2	36.2	31.2	36.2
Mean	**28.9**	**48.6**	**29.4**	**50.9**	**29.5**	**51.9**
Sd	**1.4**	**14.6**	**1.4**	**14.5**	**1.3**	**15.6**
4	31.3	51.9	31.5	52.9	31.6	53.4
1% n-3	27.9	45.3	28.5	48.4	28.6	49.0
fatty acids	30.2	51.0	31.4	57.0	31.6	58.0
+	29.3	42.9	31.2	52.2	31.1	51.7
1% FCP-3P2	29:2	49.0	29.4	50.0	29.1	48.5
	28.1	45.6	28.4	47.2	28.5	47.7
Mean	**29.3**	**47.6**	**30.1**	**51.3**	**30.1**	**51.4**
Sd	**1.3**	**3.6**	**1.5**	**3.5**	**1.5**	**3.9**

Table 1: Total free fatty acid and peroxide value in experimental diets just after (week preparation and 2-weeks later (week 2).

Diet	Total free fatty acids (% in diet sample)		Peroxide value (meq/kg diet sample	
	Week 0	Week 2	Week 0	Week 2
Control	0.45	0.35	0.79	0.2
1% n-3 fatty acids	0.44	0.41	0.92	0.7
1% n-3 fatty acids +1% FCP-3P1	0.33	0.40	0.79	0.8
1% n-3 fatty acids + 1% FCP-3P2	0.34	0.44	1.17	0.9

Table 2: Mouse body weight (g) at outset and during the experimental course

Groups (n)	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control (8)	19.9	23.2	24.7	26.3	27	27.4	27.9	28.1	28.8
1% n-3 fatty acid (8)	19.7	23.5	25.1	26.5	27.3	27.8	28.6	28.8	29.3
1% n-3 fatty acid + 1% FCP-3P1 (6)	19.7	22.6	24.4	26.5	27.2	28	28.6	28.9	29.4
1% n-3 fatty acid + 1% FCP-3P2 (6)	19.9	23.3	24.8	26.7	27.5	28.2	29	29.3	30.1

Discussion

The purpose of the above study was to examine the relationship between dietary intake of tall oil (16% sitostanol) and vegetable oil derived plant sterols (77.8% sitostanol) and omega-3 fatty acids on plasma cholesterol and triglyceride levels. Disturbances in the plasma concentration of triacylglycerol –rich lipoproteins in the postabsorptive and postprandial periods are associated with increase atherogenic risk (50). In Western population, most deaths from coronary heart disease occur

Example 1 examined relation between dietary intake of tall oil and vegetable oil derived plant sterols and omega - 3 fatty acids on plasma cholesterol and triglycerides level. The dietary plant sterol enrichment in human clinical studies consistently decreases cholesterol plasma levels but has no effect on plasma triglyceride (62). The apo E deficient mice accumulates plasma chylomicron remnants requiring apo -E for receptor uptake.

This example clearly demonstartes a significant coeffect of the combined administration of phytosterols and/or phytostanols and omega-3 PUFAs on both plasma cholesterol and triglycerides levels. The following is an analysis of the experimental results.

In this animal model there is statistically significant dietary plant sterol. omega-3 fatty acids dependent increase in plasma fasting triglyceride. As the tissue omega -3 fatty acids content increase, the plasma triglycerides levels stabilize, or start to show regression. Plant sterols decrease plasma cholesterol levels in a dose dependent manner, with the tall oil derived composition being more efficient than vegetable-derived sources. The omega - 3 fatty acids had an inconsistent non-significant inhibitory effect on plant sterol plasma cholesterol lowering, with the exception of the 0.5 % plant sterols - omega-3 fatty acids dietary supplement. where omega- 3 fatty acids suppress plant sterol cholesterol lowering effect with increase in fasting triglycerides. The plant sterol is triglyceride and omega-3 fatty acids cholesterol neutral. The study did not demonstrate any significant weight changes between the experimental groups.

There is an indication that the administration of a combination of plant sterols - omega -3 fatty acids has three phase effects: rapid accumulation of plasma triglycerides with gradual slope regression; decrease in initial cholesterol regression followed by gradual slope regression and positive correlation between cholesterol and triglyceride plasma levels. In the absence of apo E, these results are suggestive of inhibition of VLDL and chylomicron remnants catabolism due to rapid saturation of lipoprotein lipase catabolic pathway. Furthermore, omega-3 fatty acids in clinical studies decrease both plasma TG and VLDL. These arguments suggest. that omega-

Solubility of FCP-3P1 in flax and hempseed oils

This was determined by adding 100mg of the FCP-3P1 to 2mL of each of the fatty acid oils in a 10mL glass screw cap tube. Samples were equilibrated by vortexing (VWR Multi-tube Vortexer, setting 2) at either 21° C or 60° C (duplicate samples at each temperature and for each oil) for 24 hours. The tubes were allowed to attain room temperature (21° C) then centrifuged at 3000x for 5 minutes and independently sampled for analysis by gas chromatography (GC-FID), using a cholestane internal standard. Results are presented in Table 3.

Table 3: Solubility of FCP-3P1 in flax and hempola oils

Oil	FCP-3P1 (FM-PH-42) solubility (mg/mL)[a]	
	21° C	60° C*
Flax	13.79	12.07
Hempola	8.54	9.70

[a] Given as the sum of major phytosterol components (campestanol + campesterol + β-sitosterol + sitostanol = 87.85% of sample weight)

* Samples were allowed to cool to 21° C prior to analysis

It will be noted that FCP-3P1 solubility was somewhat higher in flaxseed oil than hempseed oil, at both test temperatures.

Macroemulsion formulation

A 10% w/w solution of FCP-3P1 was prepared by adding 1.5005 g of FCP-3P1 to 2.0708 g flaxseed oil and 11.4451 g soybean oil and heating to 63° C to give a clear solution. Into 10 mL of this solution was dissolved 0.7464 g of Span 60 [polyoxyethylene-(20)-sorbitan monostearate]. This constituted the oil phase. This surfactant has a Hydrophile-Lipophile Balance (HLB) value of 4.7 ± 1.0.

The aqueous phase consisted of a 15 mL solution of 0.7578 g Tween 40 [polyoxyethylene-(20)-sorbitan monopalmitate] and 0.7508 g EDTA (ethylene diamine tetra-acetic acid) in distilled de-ionized water. Tween 40 has an HLB value of 15.6 ± 1.0. Both oil and aqueous phases were individually heated to 70° C, combined and

Table 4: Content uniformity of FCP-3P1 in macroemulsion formulation

Sample #	FCP-3P1 in emulsion (mg/mL)*
1	18.95
2	19.70
3	18.68
4	19.39
5	18.52
6	18.41
Mean	18.94 (53.9% of theoretical)
Standard Deviation	0.51
Theoretical content (of test sample)	35.15

* Reflects total of major phytosterols only (campestanol + campesterol + β-sitosterol + sitostanol = 87.85% of sample weight)

Content uniformity is acceptable (18.94 ± 0.51 mg/mL) and indicates satisfactory emulsion homogeneity. Recovery is low (54%) and probably reflects a combination of incomplete extraction of active from emulsion by DCM and pipetting errors.

This dosage delivery system has successfully incorporated both FCP-3P1 and flaxseed oil (Omega fatty acid source) in a single formulation.

Example 3- Solutions and Dispersions (oil-based)

An oil-based soft gelatin capsule formulation was obtained by taking the oil phase from the macroemulsion, with or without modification, and filling the solution into a soft gelatin capsule. Potential modifications could include increasing the content of FCP-3P1, by forming a dispersion or paste; altering the ratio of FCP-3P1 (the phytosterol/stanol component) to omega fatty acids, with appropriate adjustment of the soybean oil diluent; inclusion of Tween 40 or other suitable surfactant at an appropriate level. In the event that a dispersion or paste is required, the particle size of the FCP-3P1 may be modified by milling in the dry state, or dispersed in some or all of the oil components (microfluidization), to achieve the desired end result.

10. supra, at 8

11. supra, at 9

12. Salen G. et al. J Clin. Invest. 1970; 49:952-967

13. Goodnight S.H. et al. 1982 Polyunsaturated fatty acids, hyperlipidemia and thrombosis. Atherosclerosis 2(2): 87-113

14. Sirtori C.A. Factors in formation and regression of the atherosclerotic plaque G.V.R. Born, A.L. Catapano, R. Paoletti, (eds) 163-195 Plenum Press, New York

15. Gotto A.M. et al. Nutrition, Lipids, and Coronary Heart Disease, R.I. Levy, B.M. Rifkind, B.H. Dennis, N. Ernst (eds) 247-283 Raven Press, New York

16. Grundy S.M. Dietary Fats and Sterols. In Nutrition Lipids and Coronary Heart Disease, supra at 15, 89-118

17. supra, at 13

18. supra, at 15

19. Ackman R.G. Fish Lipids. Part I. In Advances in Fish Science and technology 86-103. J.J. Connell (ed) Fishing News Books, Ltd. Farnham, Surrey, England

20. Hornstra G. The significance of fish and fish oil enriched food for prevention and therapy of cardiovascular disease. In: Vergroesen A. Crawford M. (eds) The role of fats in human nutrition. Academic Press 1989: 152-235

21. Bjerregaard P. Dyerberg J. Mortality from Ischaemic heart disease and cerebrovascular disease in Greenland. Int. J. Epidemiol. 1988; 17: 514-519

22. Ginsberg H.N. et al. J. Clin. Invest. 1986; 78:1287-95

39. supra, at 37

40. Nestel et al. J. Clin. Invest. 1984; 74:82-89

41. The Omega Connection: the facts about fish oils and human health S.K. Niazi, Health Sciences Centre. University of Illinois at Chicago, Esquire Books Inc, Chapter

42. Attwood D. Micoremulsions. In Colloidal Drug Delivery Systems (J. Kreuter ed.) Marcel Dekker, New York, 1994:32

43. Eugster C., Rivara G., Forni G and Vai S. Marigenol Concentrates comprising Taxol andor Taxan esters as active substances. Panmimerva Med, 199638:234-242

44. Liposome Drug Delivery Systems, Technomic Publishing Co. Inc., Lancaster, PA 1993

45. Pharmaceutical Technology: Liposomes as Drug Delivery Systems Parts I , II and III, October 1992, November 1992 and January 1993 respectively.

46. Rajewski R.A. and Valentino J.S. Pharmaceutical Applications of Cyclodextrins/In vivo Drug Delivery System. J. Pharm. Sci. 1996: 85:1142-1169

47. Moghadasian et al. "Tall oil"-derived phytosterol mixtures reduce atherosclerosis in apo E-deficient mice. Artherioscler Thromb Vasc Biol 1997;17:119-126.

48. Moghadasian et al. Proatherogenic and antiatherogenic effects of probucol and phytosterols in apo E-KO mice: Possible mechanism of action. Circulation 1999;99:1733-1739.

57. Connor, W.E. Hypolipidemic effects of dietary omega-3 fatty acids in normal and hyperlipidemic humans: effectiveness and mechanism. In Health effects of polyunsaturated fatty acids in sea foods. Academic Press, Orlando, Fla., Edited by A.P.Simopoulos et al.pp 173 – 210: 1986

58. Sanders, TA.B. et al. Triglycerides lowering effect of marine polyunsaturates in patients with hypertriglyceridemia. Arteriosclerosis; 50:459 – 465; 1985

59. Harris, W.S. et al. Omega - 3 fatty acids prevent carbohydrate-induced hypertriglyceridemia. Metabolism; 33:1016 – 1019; 1984

60. Clarke, S.D. et al. Dietary polyunsaturated fatty acid regulation of the gene transcription. Annu. Rev.Nutr.14: 83 – 98:1994

61. Graber R. et al. Fatty acids and cell signal transduction. J. Lipid Mediators Cell Signaling;9:91 – 116:1994

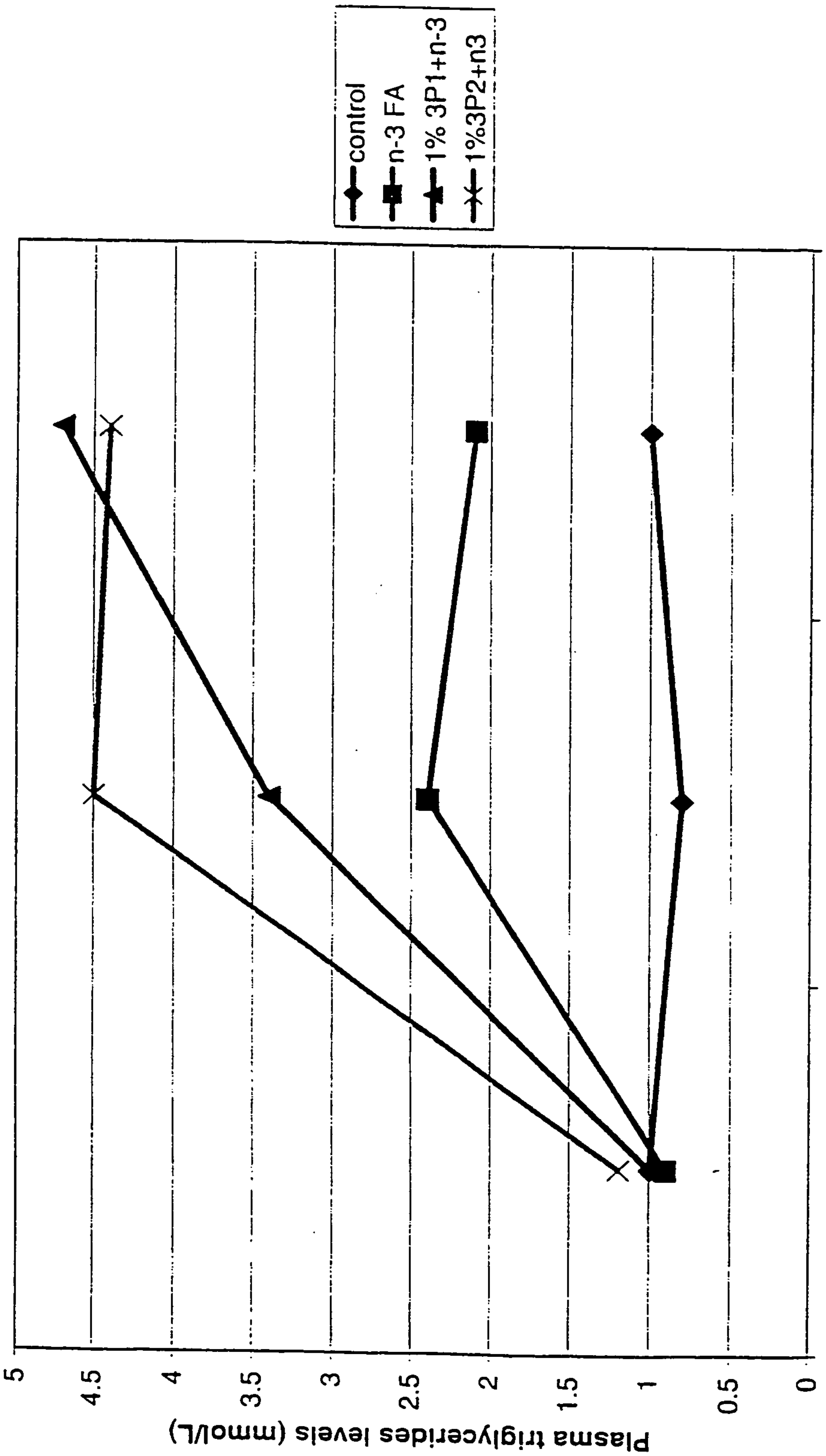
62. Miettinen T.A. et al.Regulation of cholesterol metabolism by dietary plant sterols.Current Opinion in Lipidology;10:9 – 14:1999

63. Schoonjans K. et al.The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation.Biochim. et Biophys.Acta;1302(93 – 109)1996

10. The composition of claims 1 incorporated into a vehicle selected from the group consisting of a food, a beverage, a pharmaceutical and a nutraceutical.

11. A method of treating and preventing cardiovascular disease and other disorders in an animal which comprises administering to the animal a composition comprising one or more phytosterols, phytostanols or mixtures of both, and one or more omega-3 polyunsaturated fatty acids or derivatives thereof.

Figure 2


control
n-3 FA
1% 3P1+n-3
1%3P2+n3
week 0
week 4
week 8
Plasma triglycerides levels (mmol/L)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5